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NINE ESSENTIAL AMINO ACIDS IN PURE VARIETIES OF GRAIN

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April 1949

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NINE ESSENTIAL AMINO ACIDS IN PURE VARIETIES OF GRAIN

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A THESIS
submitted to the University of Alberta
in partial fulfillment of the
requirements for the degree of
MASTER OF SCIENCE

Edmonton, Alberta
April, 1949

ACKNOWLEDGEMENTS

Sincere thanks and appreciation are extended to Dr. R. D. Sinclair, Head of the Department of Animal Science, University of Alberta, for the use of the facilities of the Department. The writer is particularly indebted to Dr. L. W. McElroy, Associate Professor of Animal Husbandry, who initiated and supervised the study herein reported, and who through helpful criticisms and suggestions rendered much valuable and appreciated assistance in carrying out this research, and in the preparation of this manuscript. The very valuable advice rendered by Dr. D. R. Clandinin, Associate Professor of Poultry Husbandry, regarding the conduction of microbiological assays is gratefully acknowledged. Thanks are also due to Mr. J. G. Wawrykow who assisted in part of the assays reported in this manuscript.

This research was made possible by a financial grant from the National Research Council, Ottawa.

TABLE OF CONTENTS

Page

Introduction 1

Review of Literature 2

Microbiological Assay Method for Amino Acids 6

Assay Materials 8

Experimental 9

        Nitrogen Content 9

        Dry Matter 9

        Hydrolysis 10

        Amino Acid Assays 10

Results and Discussion 10

        Wheat 11

        Oats 14

        Barley 16

Variations in Proportion of Amino Acids in Protein of Cereal Grains Attributable to Station and Variety Differences 23

    Wheat Protein 23

        Series A 23

            Variability due to variety 24

            Variability due to station 25

        Series B 26

            Variability due to variety 26

            Variability due to station 26

TABLE OF CONTENTS (continued)

Page

Oat Protein 27
    Variability due to variety 28
    Variability due to station 29
Barley Protein 30
    Variability due to variety 30
    Variability due to station 31
General Discussion 34
Summary 38
Literature Cited 40
Appendix - Assay Technique .... i - viii

NINE ESSENTIAL AMINO ACIDS IN PURE VARIETIES OF GRAIN

INTRODUCTION

It has been established that amino acids can be separated into two groups which are called essential and non-essential. Essential amino acids are those which cannot be synthesized by the animal concerned fast enough for optimum growth out of the materials ordinarily available in the diet. The term 'essential amino acid' has significance only when qualified as to species, nutritional state, age and purpose for which the animal is fed.

Although it is now generally recognized that adequate protein nutrition is not attained by a definite amount of protein, the majority of nutritionists and feed manufacturers appraise feeds simply on the basis of protein content as calculated from nitrogen determinations. In any attempt to evaluate the nutritive value of a feed with respect to protein, both the quantity and quality of the protein must be considered.

In considering nutritionally valuable feed proteins the first requirement is to gain as wide a knowledge as possible of the qualitative and quantitative distribution of the essential amino acids in the protein. Since cereal grains constitute a major source of dietary proteins for both humans and domestic animals, the necessity for information regarding the amino acid content of cereal grains is evident.

An estimate of the nutritive value of a protein can be made if we have a reasonably accurate picture of its amino acid

composition. However it is necessary to know the amino acid requirements of the animal, for the purpose concerned, as well as the content of the essential amino acids in the diet before we can formulate rations adequate to meet the requirements of both protein quality and quantity.

It is necessary to know the essential amino acid content of a feed in order to estimate the quantity of pure amino acids necessary to add to a ration to meet amino acid requirements and to permit the use of different proteins in the formulation of a ration so that they become mutually supplementary. The formulation of rations based on amino acid content of the ingredients will result in a great saving of money and time in both experimental feeding tests and in formulating rations.

There is a need for information regarding variability in quality of grain proteins and possible relationships between such factors as soil, climate, variety, species and the essential amino acid content of cereal grain proteins. It is with the amino acid content of wheat, oats and barley and particularly with reference to varietal and locational differences that this manuscript deals.

REVIEW OF LITERATURE

> "The current trend of the investigation of the chemistry of nutrition is emphasizing the significance of the amino acids as the fundamental factors in all problems in which hitherto the role of proteins has been involved .... Obviously the relative values of the different proteins in nutrition are based upon their content of these special amino acids which cannot be synthesized in the animal body and which are indispensable."

These statements of Osborne and Mendel (24) in 1914, cover the accepted concept of protein nutrition today. In 1906 Willcock and Hopkins (43) found the amino acid tryptophane was necessary to supplement the protein zein. Such supplementation increased its nutritive value for the growing mouse but not to as great an extent as casein. These observations of the fundamental importance of amino acids in protein nutrition were verified when Osborne and Mendel (25) published the results of their classical rat feeding experiments in 1915.

The extension of these pioneer experiments and others was brought to a successful conclusion by the well known results of W. C. Rose of Illinois (31). The program of research started in 1931 by Rose (30, 32) to determine which amino acids were essential to the growing rat and to what extent they had to be included in the diet provided proof of the essential nature for adequate nutrition of the growing rat of methionine, histidine, lysine, tryptophane, phenylalanine, threonine, leucine, isoleucine and valine. In addition the special importance of cystine, arginine, tyrosine and glycine as shown by Rose (32), Almquist and Grau (1) and others helped to pave the way to an evaluation of protein feeds, based on their comparative amino acid composition.

From a brief review of the literature, it was noted that very little work has been done to determine whether the amino acid content of any seeds varies with variety or soil and climate. In 1934 Csonka and Jones (6) reported marked variations in the cystine, tryptophane and tyrosine content of six varieties

of soybean seeds. Doty, Bergdoll, Nash and Brunson (7) in 1946 reported analyses of corn hybrids (F1 generation from inbred lines) which revealed that levels of tryptophane, tyrosine, cystine, arginine and histidine are related to the genetic constitution of the plant. Russell, Taylor, Mehrhof and Hirsch (33) in 1946 found that the availability of methionine in legumes varied with the variety, and that the nutritive value for the rat could be predicted from analyses for certain amino acids. Schweigert (34) in 1947 reported that the amino acid pattern was quite constant in samples of corn, corn gluten meal, alfalfa leaf meal, milo and soybean oil meal obtained from different sources. Sheldon, Blue and Albrecht (40) in 1948 present data illustrating wide variations in the concentrations of nine essential amino acids due to:

(a) differences between alfalfa and lespedeza hay.

(b) differences in fertility of the soil. Since protein levels were not reported in this paper it cannot be ascertained whether such differences are due to quantity or quality of the protein.

Lobay (19), on calculating mean values of percent amino acid nitrogen of total nitrogen for two varieties of oats, suggested that for the amino acids lysine, isoleucine and phenylalanine intervarietal differences may be as great as or greater than differences between species. McElroy, Clandinin, Lobay and Pethybridge (22) in 1948 from microbiological assays conducted on nine different samples of wheat, oats and barley grown under a variety of soil and climatic conditions concluded

that there were no significant correlations between soil zone and grain protein quality as measured by the proportion of nine essential amino acids in grain protein. Some variation in the proportion of total nitrogen contributed by the nitrogen of a given amino acid was noted in different samples of one variety. This locational difference was most marked for the amino acids lysine, arginine, valine and phenylalanine.

In 1949 Kuiken and Lyman (16) concluded that amino acid distribution in twenty strains of soybeans was quite uniform rather than highly variable as suggested by Csonka and Jones (6). Work in progress at this institution (27) on three varieties of field peas grown in Alberta indicates that there are no varietal differences for any of nine essential amino acids.

The literature contains many data regarding the amino acid composition of certain purified proteins and high protein feeds. The elucidation of the specific nutritive roles played by certain amino acids for growth, reproduction, lactation and maintenance indicates the need for information concerning the amino acid content of feed proteins that are commonly consumed by man and animals, rather than the knowledge of the amino acid composition of purified proteins.

Methods for determining amino acids in proteins and natural foodstuffs have been reviewed and discussed by a number of investigators (3, 5, 12). Four general methods have been used, namely chemical, physico-chemical, biological and microbiological. The development of microbiological methods for the determination of amino acids in proteins and natural foodstuffs has provided a very useful method of assay.

MICROBIOLOGICAL ASSAY METHOD FOR AMINO ACIDS

Microbiological methods, because of their specificity, accuracy, sensitivity and ability to yield many replicate results within a short time, have become of importance in the investigation of the chemistry and biochemistry of proteins and amino acids. Although such methods are of recent origin, a considerable number are already available for the assay of many of the amino acids in both purified proteins and natural products.

The microbiological method employs bacteria to assay for unknowns. A basic requirement of any bio-assay is that the nutrients known or unknown, be fed at sub-optimum levels if differences are to be detected and also that nutrients other than those under test be fed in adequate amounts.

It was not possible to develop a medium in which the only nitrogenous ingredients were specific amino acids until the amino acids were obtainable in a pure form. Kuiken and co-workers (17, 18) developed the first microbiological method of amino acid determination in 1943. The original method has been improved somewhat but the principles of most bacterimetric procedures are essentially the same.

When pure amino acids served as the only source of protein, the omission of any one of the amino acids essential for the organism was found to result in growth failure. A complete medium was made by adding minerals, vitamins and glucose in the same manner that Snell and Wright (39) employed for nicotinic acid determinations, but replacing casein with seventeen amino acids.

A medium for the determination of a particular amino acid is prepared by omitting one of the amino acids essential for the growth of the organism. To this medium a measured amount of an hydrolysate containing an unknown quantity of the amino acid is added. The medium is then inoculated with the organism and incubated at the optimum temperature. The acid produced in this culture is then titrated, the amount of base required being an indication of the growth of the organism and thus of the amount of amino acid present in the unknown.

Standards are run at the same time using different levels of the amino acid under assay and from these standard determinations, growth curves are prepared. Thus the growth curve is based on definite amounts of the amino acid under assay.

Of the amino acids recognized as physiological constituents of proteins, only three have not been reported as being essential for any organism, namely norvaline, norleucine and hydroxyproline (11). Siegel (41) reports glycine and proline in addition to the above as not yet being satisfactorily assayed. Siegel (41) reports that microbiological assays yield the most consistent and trustworthy results of any method, whereas the accuracy of the chemical determinations has been questioned since losses not only in hydrolysis, but in separation may vary significantly. This accounts for the frequently higher estimations obtained by microbiological methods. The use of the latest chemical methods in many cases produces variable results in estimating certain amino acids found in the same protein (19) whereas microbiological assay methods commonly report errors of

only 5% (26).

The degree of experimental accuracy in the amino acid analysis of the naturally occuring heterogeneous proteins, which may vary within wide limits because of preparation difficulties as well as changes in the amino acid composition of the tissue or organism itself, does not need to be as high as that required in a study of the molecular structure of proteins. Although in all analytical work one must strive for the determination of absolute values, nevertheless much valuable information can be achieved by comparative amino acid analyses, especially in relation to protein nutrition.

From early procedures using a final volume of 10 ml. a semi-micro technique was evolved which employs one ml. of the basal medium (13) and a final volume of 2 milliliters. It has been reported and verified (14, 28) that the semi-micro technique is accurate and advantageous as compared to the macro procedure.

The assays reported in this paper were conducted on a semi-micro scale in which the final volume was 2 milliliters.

ASSAY MATERIALS

Grain samples collected for a research project already in progress at the University of Alberta constituted the assay material. The materials used for assay were collected under the United Grain Growers crop testing plan for 1946. The samples used were pure varieties from definite locations, and were composite samples from four replicates grown in the trials. The samples used were not chosen to represent a wide range in protein

content or large differences in soil type. The varieties of wheat, oats and barley assayed, together with the location in which they were grown are listed in Table I.

TABLE I
Varieties of Grains Under Test

Wheat	Stations*	Oats	Stations*	Barley	Stations*
Marquis	1-3	Ajax	1-3	Newal	1-3
Red Bobs	"	Brighton	"	Sanalta	"
Redman	"	Eagle	"	Olli	"
Saunders	3-5	Exeter	"	Montcalm	"
Regent	"	Larain	"	O.A.C. 21	"
Thatcher	"	Legacy	"	Titan	"

*1 - Athabasca, 2 - Bon Accord, 3 - Sundre, 4 - Vermilion, 5 - Viking.

EXPERIMENTAL

Nitrogen Content

Nitrogen was determined on one gram samples by the Kjeldahl-Gunning-Arnold method using mercuric oxide as catalyst. Two or more determinations in duplicate were made on each sample. The percent nitrogen was calculated on a moisture-free basis. The factors used to convert nitrogen to protein were 5.7 for wheat and 6.25 for barley and oats.

Dry Matter

Dry matter was determined by heating two grams of finely ground sample in a force draft oven for 18 hours at 105°C. Two or more determinations in duplicate were made on each sample.

Hydrolysis

Hydrolysis was carried out in 250 ml. Erlenmeyers covered with 50 ml. beakers. One gram of sample and 25 ml. of 2 N HCl were placed in each Erlenmeyer. Autoclaving for 10 hours was adopted as a routine procedure.

Amino Acid Assays

Nine essential amino acids were determined microbiologically on the hydrolysates with the following organisms for the amino acids indicated: Leuconostoc mesenteroides P-60, lysine (8); Lactobacillus arabinosus 17-5, leucine,(36), isoleucine (37), phenylalanine (28) and valine (37); Streptococcus faecalis R, methionine (29), arginine (42), histidine (42), and threonine (10). The details of the procedure employed in the assays are outlined in the appendix.

RESULTS AND DISCUSSION

In this study amounts of nine essential amino acids in six varieties each of wheat, oats and barley were determined. A total of eighteen samples of each grain, obtained from three locations in Alberta, were assayed. Assay values listed for each variety and each station in Tables II - IV represent the mean of at least four separate assays. Each assay was done on a different hydrolysate and represents the mean value for three levels of the unknown sample.

The values reported were determined in micrograms, converted to milligrams per gram sample and reported as percent

amino acid nitrogen of total nitrogen on a moisture-free basis. This was done in order to offset any variations in the level of nitrogen between samples assayed. The following method was used to calculate the individual results to this basis.

$$\text{Mg. amino acid per gram} \quad \text{X} \quad \frac{1000}{\text{Mg. dry matter per gram}} \quad \text{X} \quad \frac{100}{\text{Mg. N per gram}} \quad \text{X} \quad \frac{\text{Amino acid N}}{\text{Mol. wt. of amino acid}}$$

Wheat

The results of assays done on the six varieties of wheat studied are presented in Table II.

TABLE II

Nine Essential Amino Acids in Six Varieties of Wheat Grown in Alberta.

(Data are reported on a moisture-free basis)

			% Amino Acid N of Total N								
Variety	Station	Nitrogen %	Lysine	Leucine	Isoleucine	Phenylalanine	Valine	Methionine	Arginine	Histidine	Threonine
Thatcher	Vermilion	3.09	2.86	3.90	2.69	2.42	3.00	.77	9.11	3.14	1.91
"	Viking	2.95	2.85	4.02	2.75	2.30	2.98	.83	8.43	3.06	1.78
"	Sundre	1.99	3.39	3.88	2.98	2.35	3.30	.89	9.25	3.39	2.01
	Mean	2.68	3.03	3.93	2.81	2.36	3.09	.83	8.93	3.20	1.90
Regent	Vermilion	3.02	2.87	4.05	2.89	2.63	2.96	.77	9.20	3.13	1.83
"	Viking	2.94	2.70	4.07	2.83	2.38	3.24	.80	8.60	3.05	1.79
"	Sundre	2.05	3.06	4.12	2.87	3.12	3.18	.86	8.16	3.06	1.86
	Mean	2.67	2.88	4.08	2.86	2.71	3.13	.81	8.65	3.08	1.83
Saunders	Vermilion	3.04	2.97	4.15	2.80	2.47	3.08	.85	9.64	3.14	2.00
"	Viking	2.69	3.14	4.20	2.94	2.43	3.16	.85	9.83	3.11	1.87
"	Sundre	1.95	3.55	4.08	2.90	2.55	3.15	.87	8.54	2.70	2.03
	Mean	2.56	3.22	4.14	2.88	2.48	3.13	.86	9.34	2.98	1.97
Marquis	Athabasca	2.63	2.54	4.13	3.09	2.20	3.31	.77	8.82	2.75	1.97
"	Bon Accord	2.07	3.05	4.19	2.87	2.28	3.50	.84	9.61	2.94	2.06
"	Sundre	1.98	3.21	4.18	3.28	2.14	3.60	.86	9.50	2.68	2.15
	Mean	2,23	2.93	4.17	3.08	2.21	3.47	.82	9.31	2.79	2.06
Redman	Athabasca	2.62	2.60	4.26	3.21	2.44	3.32	.78	8.71	2.66	1.57
"	Bon Accord	2.00	2.83	4.12	3.28	2.21	3.40	.83	9.15	2.88	1.95
"	Sundre	1.95	2.90	4.13	3.31	2.18	3.54	.84	8.69	2.98	2.00
	Mean	2.19	2.78	4.17	3.27	2.28	3.42	.82	8.85	2.84	1.84
Red Bobs	Athabasca	2.51	2.63	4.14	3.12	2.29	3.27	.79	9.09	2.61	2.01
"	Bon Accord	1.88	2.98	4.14	3.22	2.18	3.52	.85	9.34	2.79	2.04
"	Sundre	1.78	3.17	4.19	3.26	2.22	3.50	.94	9.47	2.88	2.24
	Mean	2.06	2.93	4.16	3.20	2.23	3.43	.86	9.30	2.76	2.10
	Mean, 6 varieties	2.40	2.96	4.11	3.02	2.38	3.28	.83	9.06	2.94	1.95

All varieties of wheat are of the hard spring wheat type and are grown commercially in Alberta. An attempt was made to obtain samples of all six varieties of wheat, oats and barley grown at Athabasca, Bon Accord and Sundre, but as is evident from Table II this was not possible for the wheat varieties Thatcher, Regent and Saunders. Samples in Table II are listed in order of decreasing nitrogen content for both varieties and stations.

It may be noted that in each variety there is a trend towards an increase in per cent lysine nitrogen of total nitrogen with decreasing levels of nitrogen. A correlation coefficient for lysine N as per cent of total N was established at — .530 for the data presented in Table II. This coefficient was found to be significant at the 5% point by the t test (9).

This agrees with the results of McElroy, Clandinin, Lobay and Pethybridge (22) who report a trend towards a decrease in per cent lysine nitrogen of total nitrogen with increasing levels of total nitrogen, in nine samples of Marquis wheat selected to cover a wide range in protein content and to represent grains grown under a variety of soil and climatic conditions. The values reported by Schweigert (35) for lysine in five samples of wheat of different protein content also suggest the possibility of an inverse relationship between total protein and per cent lysine of protein.

It is recognized (2) that the total nitrogen content of grains is affected by the conditions of soil and climate under which they are grown; for example, it is evident from Table II

that the samples of each variety grown at Sundre contained less nitrogen than those grown at other stations.

Oats

The results of assays expressed as per cent amino acid nitrogen of total nitrogen in six varieties of oats are presented in Table III.

TABLE III

Nine Essential Amino Acids in Six Varieties of Oats Grown in Alberta

(Data are reported on a moisture-free basis)

Variety	Station	Nitrogen %	% Amino Acid N of Total N								
			Lysine	Leucine	Isoleucine	Phenylalanine	Valine	Methionine	Arginine	Histidine	Threonine
Larain	Athabasca	2.75	3.68	4.55	2.81	1.60	3.89	.56	12.34	2.42	2.22
"	Bon Accord	2.25	3.35	4.38	2.96	1.53	3.84	.53	11.27	2.43	2.16
"	Sundre	2.24	3.32	4.09	2.67	1.78	3.68	.53	11.37	2.50	2.21
	Mean	2.41	3.45	4.34	2.81	1.64	3.80	.54	11.66	2.45	2.20
Brighton	Athabasca	2.73	5.05	4.40	2.98	2.52	4.24	.74	12.61	2.60	2.17
"	Bon Accord	1.95	5.34	4.39	3.28	2.43	4.51	.82	13.49	2.85	2.42
"	Sundre	1.76	5.30	4.30	3.29	2.35	4.37	.79	11.81	2.68	2.10
	Mean	2.15	5.23	4.36	3.18	2.43	4.37	.77	12.64	2.71	2.23
Legacy	Athabasca	2.50	3.26	3.97	2.71	1.36	3.58	.51	10.90	2.40	2.26
"	Sundre	1.85	3.49	4.19	2.99	1.40	3.74	.56	12.22	2.27	2.26
"	Bon Accord	1.83	3.26	4.00	3.03	1.50	3.64	.56	11.46	2.37	2.24
	Mean	2.06	3.34	4.05	2.91	1.42	3.65	.54	11.53	2.35	2.25
Ajax	Athabasca	2.40	4.16	3.98	3.10	2.45	4.24	.66	11.95	2.19	1.95
"	Sundre	1.81	4.99	4.42	3.28	2.30	4.32	.69	10.54	2.18	2.01
"	Bon Accord	1.78	4.26	4.06	3.24	2.35	4.31	.66	11.07	2.32	1.85
	Mean	2.00	4.47	4.15	3.21	2.37	4.29	.67	11.19	2.23	1.94
Exeter	Athabasca	2.42	3.34	4.14	2.87	1.27	3.48	.66	11.89	2.31	1.98
"	Bon Accord	1.84	3.63	3.97	3.02	1.75	3.50	.57	11.89	2.46	2.21
"	Sundre	1.70	3.57	3.83	2.83	1.68	3.61	.57	11.34	2.37	2.07
	Mean	1.99	3.51	3.98	2.91	1.57	3.53	.60	11.71	2.38	2.09
Eagle	Athabasca	2.01	4.71	4.12	3.11	2.28	4.43	.68	12.14	2.38	2.09
"	Bon Accord	1.66	5.69	4.18	3.21	2.33	4.25	.71	11.49	2.40	1.92
"	Sundre	1.38	5.52	4.10	3.40	2.23	4.31	.70	12.24	2.71	2.10
	Mean	1.68	5.31	4.13	3.24	2.28	4.33	.70	11.96	2.50	2.04
	Mean, 6 varieties	2.05	4.22	4.17	3.04	1.95	4.00	.64	11.78	2.44	2.12

All varieties of oats listed in Table III are grown commercially in Alberta. Brighton oats is the only variety of the hulless type assayed in this study. All varieties of oats were grown at each of three stations. Samples in Table III are listed in order of decreasing nitrogen content for both varieties and stations. For each variety samples grown at Athabasca contained the highest level of nitrogen. Excepting Larain oats the highest levels of nitrogen, in the samples listed in Table III, are accompanied by the lowest proportion of lysine nitrogen of total nitrogen. A correlation coefficient of – .392, significant at the 5% point, was established for per cent lysine nitrogen of total nitrogen in the samples of oats reported in Table III.

Barley

The results of assays done on six varieties of barley are summarized in Table IV. Each variety is commonly grown in Alberta. The varieties Newal, Sanalta and Titan are feed barleys whereas the varieties Montcalm, O.A.C. 21 and Olli are acceptable to the malting trade. The latter is the only rough-awned barley listed in Table IV. The barley samples are listed in order of decreasing nitrogen content for both varieties and stations.

TABLE IV

Nine Essential Amino Acids in Six Varieties of Barley Grown in Alberta

(Data are reported on a moisture-free basis)

Variety	Station	Nitrogen %	% Amino Acid N of Total N								
			Lysine	Leucine	Isoleucine	Phenyl-alanine	Valine	Methionine	Arginine	Histidine	Threonine
Newal	Bon Accord	2.20	3.32	3.87	2.79	2.30	3.36	.80	7.94	2.40	2.16
"	Athabasca	2.04	3.46	3.79	2.80	2.26	3.37	.78	8.02	2.31	2.02
"	Sundre	1.87	3.40	3.89	2.87	2.10	3.40	.81	8.40	2.41	2.13
	Mean	2.04	3.39	3.85	2.82	2.22	3.38	.80	8.12	2.37	2.10
Sanalta	Athabasca	2.05	3.23	3.80	2.84	2.14	3.42	.80	8.09	2.33	1.90
"	Bon Accord	1.93	3.28	3.83	2.96	2.17	3.41	.81	8.32	2.49	2.03
"	Sundre	1.86	3.37	3.76	2.81	2.05	3.30	.81	8.64	2.47	1.94
	Mean	1.95	3.29	3.80	2.87	2.12	3.38	.81	8.35	2.43	1.96
Titan	Athabasca	2.13	3.35	4.25	2.54	2.32	3.92	.95	9.15	3.15	2.32
"	Bon Accord	1.96	3.53	4.30	2.57	2.59	4.33	1.10	11.12	3.64	3.25
"	Sundre	1.72	4.15	4.48	2.73	2.48	3.84	1.18	9.75	3.17	2.72
	Mean	1.94	3.68	4.34	2.61	2.46	4.03	1.08	10.01	3.32	2.76
Olli	Athabasca	1.95	3.70	3.95	2.72	2.07	3.40	.83	9.09	2.34	2.10
"	Bon Accord	1.88	3.63	3.76	2.68	1.96	3.28	.79	9.14	2.46	1.86
"	Sundre	1.74	3.75	3.90	2.79	1.97	3.32	.81	8.39	2.45	2.00
	Mean	1.86	3.69	3.87	2.73	2.00	3.33	.81	8.87	2.42	1.99
O.A.C. 21	Athabasca	1.97	3.78	4.08	2.48	2.33	3.91	.97	10.73	2.74	2.43
"	Bon Accord	1.84	3.65	4.18	2.50	2.29	3.89	.97	9.81	2.89	2.36
"	Sundre	1.60	4.49	4.18	2.62	2.33	4.17	1.04	10.23	2.88	2.64
	Mean	1.80	3.97	4.15	2.53	2.32	3.99	.99	10.26	2.84	2.48
Montcalm	Athabasca	1.76	3.94	4.23	2.66	2.44	3.96	1.03	10.70	3.13	2.72
"	Sundre	1.67	4.21	4.40	2.65	2.32	3.75	1.08	10.25	2.96	2.54
"	Bon Accord	1.63	4.28	4.03	2.57	2.58	3.81	.98	10.87	3.03	2.53
	Mean	1.69	4.14	4.22	2.63	2.45	3.84	1.03	10.61	3.04	2.60
	Mean, 6 varieties	1.88	3.69	4.04	2.70	2.26	3.66	.93	9.37	2.74	2.32

By inspection of Table IV it can be seen that the three malting barleys have a lower total nitrogen content than the three feed varieties. In all varieties except Newal the highest level of nitrogen is to be found in those samples obtained from the Athabasca station. Excepting Montcalm, the varieties listed in Table IV exhibit the lowest levels of nitrogen in those samples obtained from Sundre. Excepting the varieties Newal and Montcalm the proportion of lysine nitrogen is lowest in the barley grown at Sundre. A correlation coefficient of —.845, significant at the 1% point, was calculated for the per cent lysine nitrogen of total nitrogen in the eighteen samples of barley listed in Table IV.

When the results reported in Tables II-IV are totaled they reveal that the average of six varieties was: wheat 30.84%, oats 34.36% and barley 31.71%. This agrees essentially with the results reported by McElroy, Clandinin, Lobay and Pethybridge (22) who found the corresponding values in one variety of each grain to be: wheat 30.17%, oats 36.00% and barley 31.98%.

From inspection of Tables II-IV it can be noted that there are marked differences in the nitrogen content of samples between varieties and stations. In order to ascertain whether or not the nitrogen levels are dependent on the variety and the station, statistical analyses were conducted on the original data. A summary of the complex analyses of variance used to establish probabilities is listed in Table V.

TABLE V
Complex Analyses of Variance of Percent Nitrogen in Wheat, Oats and Barley

Variance due to	Wheat(1) D.F.	Wheat(1) Mean Square	Oats D.F.	Oats Mean Square	Barley D.F.	Barley Mean Square
Variety	2	.101**	5	.719**	5	.265**
Station	2	1.673**	2	3.494**	2	.323**
Variety x Station	4	.002	10	.073**	10	.024**
Replicates in General	9	.001	9	.002	9	.002
Error	18	.0012	45	.0041	45	.0025
Total	35		71		71	

**Significant at the 1% level (highly significant).

(1) Statistical analysis of per cent nitrogen in wheat refers to Marquis, Red Bobs and Redman varieties grown at Athabasca, Bon Accord and Sundre.

By inspection of Table V it can be seen that there are highly significant differences in per cent nitrogen between both varieties and stations in the samples of wheat, oats and barley listed in Tables II-IV.

From the data presented in Table V, minimum significant differences were calculated. When these were applied to the means listed in Tables II-IV they revealed the probabilities of the existence of differences in nitrogen levels in each grain with respect to both station and variety. The probabilities for which the odds were found to exceed 100:1 in favor of varietal differences are listed in Table VI.

TABLE VI
Probability of Inter Varietal Differences in Three Grains

Wheat[1]

Marquis exceeds Red Bobs in per cent nitrogen

Oats

Larain	exceeds	Brighton	in	per	cent	nitrogen
"	"	Legacy	"	"	"	"
"	"	Ajax	"	"	"	"
"	"	Exeter	"	"	"	"
"	"	Eagle	"	"	"	"
Brighton	"	"	"	"	"	"
Legacy	"	"	"	"	"	"
Ajax	"	"	"	"	"	"
Exeter	"	"	"	"	"	"

Barley

Newal	exceeds	Olli	in	per	cent	nitrogen
"	"	O.A.C. 21	"	"	"	"
"	"	Montcalm	"	"	"	"
Sanalta	"	O.A.C. 21	"	"	"	"
"	"	Montcalm	"	"	"	"
Titan	"	O.A.C. 21	"	"	"	"
"	"	Montcalm	"	"	"	"
Olli	"	"	"	"	"	"

---

[1] Statistical analysis of per cent nitrogen in wheat refers to Marquis, Red Bobs and Redman varieties grown at Athabasca, Bon Accord and Sundre.

The probabilities for which odds were found to be greater than 100:1 in favor of a station difference are presented in Table VII for each grain.

TABLE VII
Probability of Inter Station Differences in Three Grains

Athabasca	exceeds	Bon Accord	in	per	cent	N	in	wheat(1)
"	"	Sundre	"	"	"	"	"	"
Bon Accord	"	"	"	"	"	"	"	"
Athabasca	"	"	"	"	"	"	"	oats
"	"	"	"	"	"	"	"	barley
Bon Accord	"	"	"	"	"	"	"	"

---

(1) N levels at only Athabasca, Bon Accord and Sundre were analyzed statistically.

By inspection of Table VII it can be seen that in all three species the nitrogen content of samples grown at Athabasca was significantly higher than that of samples grown at Sundre, and that the nitrogen content of samples grown at Bon Accord tended to be intermediate.

These data, which indicate the existence of highly significant variations in total nitrogen content of grains attributable to both variety and environmental factors characteristic of the location in which the grains may be grown, are in agreement with results reported by McCalla and Rose (21) for wheat and by Neatby and McCalla (23) for wheat and barley.

It has been established that there is a negative correlation between per cent lysine nitrogen of total nitrogen and total nitrogen which is significant at the 5% point for the samples of wheat and oats assayed, and at the 1% point for the barley samples. This is in agreement with McElroy, Clandinin, Lobay and Pethybridge (22) who reported that negative trends were observed in lysine nitrogen as per cent of total nitrogen in wheat and barley. They found that the variation existing

in the per cent of the total nitrogen contributed by the nitrogen of a given amino acid in different samples of one variety appears to be most marked for the amino acids lysine, arginine, valine and phenylalanine.

The study reported herein was conducted on six varieties each of wheat, oats and barley. Microbiological assays for nine essential amino acids were carried out a sufficient number of times on each sample to establish whether the differences associated with varieties or with stations were statistically significant. The greatest variability appears to exist in the proportion of lysine, valine and arginine in the proteins of each grain listed in Tables II-IV. In the following section of this manuscript statistical interpretations of the observed variabilities, with respect to these three amino acids, are presented.

VARIATIONS IN PROPORTION OF AMINO ACIDS IN PROTEIN OF CEREAL GRAINS ATTRIBUTABLE TO STATION AND VARIETY DIFFERENCES

WHEAT PROTEIN

The data for per cent amino acid nitrogen of total nitrogen in the wheat samples reported in Table II were studied statistically for the three amino acids lysine, valine and arginine. A complex analysis of variance (9) was used to ascertain whether the variation due to variety or stations was significant. Two separate series for the wheat data summarized in Table II were set up and analyzed. Series A includes the varieties Marquis, Red Bobs and Redman grown at three stations, Athabasca, Bon Accord and Sundre. Series B consists of the varieties Saunders, Regent and Thatcher grown at Vermilion, Viking and Sundre.

Series

Series A

The complex analyses of variance for per cent lysine, valine and arginine nitrogen of total nitrogen in the wheat samples studied in Series A are presented in Table VIII.

TABLE VIII
Complex Analyses of Variance for Per Cent of Amino Acid Nitrogen of Total Nitrogen in Nine Samples of Wheat - Series A.

Variance due to	Lysine D.F.	Lysine Mean Square	Valine D.F.	Valine Mean Square	Arginine D.F.	Arginine Mean Square
Variety	2	.228	2	.017	2	1.476**
Station	2	1.500**	2	.330*	2	1.274**
Variety x Station	4	.067**	4	.018	4	.305
Replicates in general	15	.013	15	.077	15	.427
Error	30	.0153	30	.0075	30	.1867
Total	53		53		53	

* Significant at the 5% level (significant).
**Significant at the 1% level (highly significant).

From inspection of Table VIII it can be seen that there are highly significant differences, between varieties, in the proportion of arginine in the protein of the wheat samples included in Series A. The proportion of valine in the total protein varied significantly between stations and the station differences for lysine and arginine were highly significant.

Variation due to variety

The complex analyses of variance presented in Table VIII reveal highly significant differences in the proportion of arginine in the protein of the three varieties. The specific comparisons made, for which the odds are greater than 100:1 in favor of variety differences, are listed in Table IX.

TABLE IX
Probability of Inter Varietal Differences in Wheat

Marquis exceeds Redman in percent arginine N of total N
Red Bobs " " " " " " " " "

From Table IX it can be readily noted that the proportion of arginine in the protein of both Marquis and Red Bobs was found to be higher than that determined in Redman wheat.

Variability due to stations

By calculating minimum significant differences from the data presented in Table VIII it was found that the means for three varieties grown at one station differed significantly from those for the same varieties grown at another station. The probabilities of specific station differences in the proportion of nitrogen contributed by the nitrogen of each of the three amino acids are presented in Table X.

TABLE X
Probability of Inter Station Differences in Wheat

	Probability					Odds in favor
Sundre	exceeds	Athabasca	in per cent	lysine	N of total N	>100:1
Bon Accord	"	"	" " "	"	" " " "	> "
Sundre	"	Bon Accord	" " "	valine	" " " "	> 20:1
"	"	Athabasca	" " "	"	" " " "	> "
Bon Accord	"	"	" " "	"	" " " "	> "
"	"	"	" " "	arginine	" " " "	>100:1

From Table X it can be concluded that the protein of wheat samples grown at Sundre contained a higher proportion of lysine and valine than did the protein of samples from Athabasca. Wheat samples obtained from Sundre contained a higher proportion of valine in their protein than samples from Bon Accord. Samples of wheat obtained from Bon Accord contained a higher proportion of lysine, valine and arginine in their protein than was found in the samples from Athabasca.

Series B

The complex analyses of variance of per cent lysine, valine and arginine nitrogen of total nitrogen in the wheat samples studied in Series B are presented in Table XI.

TABLE XI
Complex Analyses of Variance for Per Cent of Amino Acid Nitrogen of Total Nitrogen in Nine Samples of Wheat - Series B.

Variance due to	Lysine D.F.	Lysine Mean Square	Valine D.F.	Valine Mean Square	Arginine D.F.	Arginine Mean Square
Variety	2	.151	2	.026	2	.701
Station	2	.483*	2	.063	2	2.864
Variety x Station	4	.062**	4	.140**	4	2.376**
Replicates in general	9	.037	9	.010	9	.112
Error	18	.004	18	.0092	18	.2446
Total	35		35		35	

*Significant at the 5% level (significant).
**Significant at the 1% level (highly significant).

Variability due to variety

No statistical significances in the proportions of lysine, valine or arginine in wheat protein were found between the varieties studied in Series B.

Variability due to stations

By inspection of Table XI it can be seen that a significant difference was found between stations in the case of the amino acid lysine. The probabilities, for which the odds are greater than 20:1 in favor of station differences, are listed in Table XII.

TABLE XII
Probability of Inter Station Differences in Wheat

Sundre exceeds Vermilion in per cent lysine N of total N
" " Viking " " " " " " " "

As shown in Table XII, the minimum significant differences calculated for lysine revealed that on the average the protein of samples of Saunders, Thatcher and Regent wheat grown at Sundre contained a significantly higher proportion of lysine than did the protein of samples of the same varieties grown at Viking or Vermilion.

OAT PROTEIN

The original data for per cent amino acid nitrogen of total nitrogen in eighteen samples of oats, summarized in Table III, were studied statistically for three amino acids, lysine, valine and arginine.

Complex analyses of variance (9) were used to ascertain whether the variations due to variety or stations were significant. Unlike wheat the statistical analyses of the results for oats were carried out to compare six varieties grown at each of three stations; Athabasca, Bon Accord and Sundre.

A summary table of the statistics obtained is presented in Table XIII.

TABLE XIII
Complex Analyses of Variance of Percent
Amino Acid Nitrogen of Total Nitrogen
in Eighteen Samples of Oats

Variance due to	Lysine D.F.	Lysine Mean Square	Valine D.F.	Valine Mean Square	Arginine D.F.	Arginine Mean Square
Variety	5	10.22**	5	3.757**	5	2.277
Station	2	1.034	2	.013	2	.184
Variety x Station	10	.390*	10	.051	10	1.037**
Replicates in general	9	.385	9	.230**	9	.102
Error	45	.1572	45	.050	45	.235
Total	71		71		71	

*Significant at the 5% level (significant).
**Significant at the 1% level (highly significant).

Variability due to variety

From Table XIII it can be seen that there are highly significant differences, between varieties, in the proportion of lysine and valine found in oat protein. Individual comparisons using minimum significant differences calculated from the analyses of variance summarized in Table XIII and applied to the means of the varieties listed in Table III reveal certain varietal differences. The probabilities as listed in Table XIV have odds, in favor of a varietal difference, in excess of 100:1.

TABLE XIV

Probability of Inter Varietal Differences in Oats

Eagle	exceeds	Ajax	in	per	cent	lysine	N	of	total	N
"	"	Exeter	"	"	"	"	"	"	"	"
"	"	Larain	"	"	"	"	"	"	"	"
"	"	Legacy	"	"	"	"	"	"	"	"
Brighton	"	Ajax	"	"	"	"	"	"	"	"
"	"	Exeter	"	"	"	"	"	"	"	"
"	"	Larain	"	"	"	"	"	"	"	"
"	"	Legacy	"	"	"	"	"	"	"	"
Ajax	"	Exeter	"	"	"	"	"	"	"	"
"	"	Larain	"	"	"	"	"	"	"	"
"	"	Legacy	"	"	"	"	"	"	"	"
Brighton	"	Legacy	"	"	"	valine	"	"	"	"
"	"	Exeter	"	"	"	"	"	"	"	"
Eagle	"	Legacy	"	"	"	"	"	"	"	"
"	"	Exeter	"	"	"	"	"	"	"	"
Ajax	"	Legacy	"	"	"	"	"	"	"	"
"	"	Exeter	"	"	"	"	"	"	"	"

With reference to Table XIV it can be seen that there are many differences in the proportion of both lysine and valine in the protein of the oat varieties listed in Table III. Both Brighton and Eagle oats contain a greater proportion of lysine and valine in their protein than is found in Legacy or Larain. Brighton, the only hulless oat variety assayed, did not differ, at the 1% level, from Eagle in per cent lysine, or from Eagle, Ajax or Larain in per cent valine of protein.

Variability due to station

From Table XIII it can be noted that there are no significant differences due to stations in the proportion of lysine, valine or arginine in the protein of the oat samples.

BARLEY PROTEIN

The assay results for lysine, valine and arginine in barley were analyzed by the same method as those for oats. The analyses of variance of the data for six varieties of barley, grown at each of three stations, are summarized in Table XV.

TABLE XV
Complex Analyses of Variance of Percent
Amino Acid Nitrogen of Total Nitrogen
in Eighteen Samples of Barley

	Lysine		Valine		Arginine	
Variance due to	D.F.	Mean Square	D.F.	Mean Square	D.F.	Mean Square
Variety	5	1.342**	5	1.310**	5	23.484**
Station	2	1.016**	2	.094	2	.582
Variety x Station	10	.259	10	.054	10	.964*
Replicates in general	9	.501**	9	.230	9	.529
Error	45	.144	45	.0537	45	.4691
Total	71		71		71	

Variability due to variety

From inspection of Table XV it can be noted that there are highly significant differences due to varieties in the proportion of barley protein contributed to by each of lysine, valine and arginine.

With minimum significant differences calculated from Table XV the specific comparisons, listed in Table XVI, having odds greater than 100:1 in favor of varietal differences were made.

TABLE XVI

Probability of Inter Varietal Differences in Barley

Montcalm	exceeds	Olli	in	percent	lysine	N	of	total	N
"	"	Titan	"	"	"	"	"	"	"
"	"	Newal	"	"	"	"	"	"	"
"	"	Sanalta	"	"	"	"	"	"	"
O.A.C. 21	"	Newal	"	"	"	"	"	"	"
"	"	Sanalta	"	"	"	"	"	"	"
Olli	"	"	"	"	"	"	"	"	"
Titan	"	"	"	"	"	"	"	"	"
"	"	Newal	"	"	valine	"	"	"	"
"	"	Sanalta	"	"	"	"	"	"	"
"	"	Olli	"	"	"	"	"	"	"
O.A.C. 21	"	Newal	"	"	"	"	"	"	"
"	"	Sanalta	"	"	"	"	"	"	"
"	"	Olli	"	"	"	"	"	"	"
Montcalm	"	Newal	"	"	"	"	"	"	"
"	"	Sanalta	"	"	"	"	"	"	"
"	"	Olli	"	"	"	"	"	"	"
"	"	"	"	"	arginine	"	"	"	"
"	"	Sanalta	"	"	"	"	"	"	"
"	"	Newal	"	"	"	"	"	"	"
O.A.C. 21	"	Olli	"	"	"	"	"	"	"
"	"	Sanalta	"	"	"	"	"	"	"
"	"	Newal	"	"	"	"	"	"	"
Titan	"	Olli	"	"	"	"	"	"	"
"	"	Sanalta	"	"	"	"	"	"	"
"	"	Newal	"	"	"	"	"	"	"

From Table XVI it can be seen that Titan and two of the malting varieties, Montcalm and O.A.C. 21, contain the highest proportions of the three amino acids, lysine, valine and arginine, in their proteins. Olli barley, the third malting variety, contains the lowest proportion of valine of any of the varieties listed.

Variability due to station

With reference to Table XV it can be noted that the variance due to station is highly significant for the proportion of lysine in barley protein. By calculating a

minimum significant difference the probabilities, for which odds are greater than 100:1 in favor of station differences, are listed in Table XVII.

TABLE XVII
Probability of Inter Station Differences in Barley

Sundre exceeds Athabasca in per cent lysine N of total N
" " Bon Accord " " " " " " " "

With reference to Tables II and IV the proportion of lysine in both wheat and barley proteins has been proven to be highest in samples obtained from Sundre. In the wheat samples of Series A the proportion of lysine in samples from Sundre and Bon Accord is greater than in those samples grown at Athabasca.

The wheat samples in Series B grown at Sundre contained a higher proportion of lysine in their proteins than did those from either Vermilion or Viking.

The protein of the barley samples grown at Sundre was higher than in the protein of barleys obtained from either Athabasca or Bon Accord. In addition the wheat samples in Series A from Sundre contained a higher proportion of lysine in their protein than did samples grown at either Bon Accord or Athabasca.

The protein of wheat obtained from Bon Accord contained a higher proportion of both valine and arginine than did samples obtained from Athabasca. In contrast to the results obtained for wheat and barley, the data for oats do not indicate the existence of significant differences attributable to stations,

in the proportion of any of the three essential amino acids studied in oat protein.

With reference to varietal differences it has been shown that in Table II the proportion of arginine in the protein of both Marquis and Red Bobs wheat is significantly higher than that in Redman.

It has been demonstrated that in Table III the proportion of lysine in the protein of Eagle and Brighton oats exceeds that of Ajax, Exeter, Larain and Legacy. The proportion of valine in the protein of Brighton, Eagle and Ajax oats was higher than that of Legacy or Exeter.

With reference to Table IV the proportion of lysine in the protein of Montcalm barley exceeds that in Olli, Titan, Newal or Sanalta. O.A.C. 21 was found to contain a higher proportion of lysine in its protein than was found in Newal or Sanalta. Olli and Titan barleys exceeded Sanalta in per cent lysine of total protein. The proportion of both valine and arginine in the protein of Titan, O.A.C. 21 and Montcalm was greater than that found in the Newal, Sanalta or Olli varieties.

Forty-five individual comparisons have been made which carry odds greater than 100:1 in favor of the existence of varietal differences with respect to the proportion of certain amino acids in grain protein.

GENERAL DISCUSSION

Analyses of the data summarized in Tables II-IV have shown that significant differences may exist, between varieties and between stations, in the quality of cereal grain proteins as measured by the proportion of the total nitrogen that is contributed by the nitrogen of individual essential amino acids, notably lysine, valine and arginine. Thus, for example, it was shown in Table XVI that the proteins of the three malting varieties of barley, Montcalm, O.A.C. 21 and Olli, tend to contain a higher proportion of lysine than the proteins of Titan, Sanalta and Newal which are grown for feed. The data in Tables II-IV also suggest the possibility of species differences in the amino acid composition of the protein of cereal grains and it was noted that the average percentage of the total nitrogen contributed by the nitrogen of the nine amino acids under study was 34.36, 31.71 and 30.84 for 6 varieties of oats, barley and wheat respectively.

These examples suggest that the proteins of the malting varieties of barley may be of better quality than those of the feeding varieties in that they contain a higher proportion of the amino acid lysine which is required in relatively large amounts in animal rations, and that the proteins of oats may be of better quality than those of barley or wheat as measured by the proportion of the total nitrogen which can be traced to the nitrogen of nine essential amino acids. Conclusions of this nature, although possibly correct technically, may be

somewhat misleading from the practical point of view in that they do not take into account differences in total nitrogen content which are characteristic of species, of varieties within a species and of the environment in which the grains are grown. With these points in mind it is of interest to compare the data in Tables II-IV, in which results are summarized in terms of percent amino acid nitrogen of total nitrogen, with those in Table XVIII in which the assay results are expressed in terms of percent amino acid of whole grain.*

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*Formula for conversion:

$$\frac{\%\ \text{amino acid N of total N}}{100} \times \%\ \text{N} \times \frac{\text{M.W. amino acid}}{\text{Wt. amino acid N}} = \begin{array}{l}\%\ \text{amino acid}\\ \text{of whole grain}\end{array}$$

TABLE XVIII
Amino Acid Content of Cereal Grains

(Data are reported on a moisture-free basis)

		% amino acid in whole grain									
Variety	Protein %	Lysine	Leucine	Isoleucine	Phenylalanine	Valine	Methionine	Arginine	Histidine	Threonine	Total
Wheat											
Thatcher	15.28	0.42	0.99	0.70	0.75	0.69	0.24	0.74	0.32	0.43	5.28
Regent	15.22	0.40	1.02	0.72	0.85	0.70	0.23	0.72	0.30	0.42	5.36
Saunders	14.59	0.43	0.99	0.69	0.75	0.67	0.23	0.74	0.28	0.43	5.21
Marquis	12.71	0.34	0.87	0.64	0.58	0.65	0.19	0.65	0.23	0.39	4.54
Redman	12.48	0.32	0.85	0.67	0.59	0.63	0.19	0.60	0.23	0.34	4.42
Red Bobs	11.74	0.32	0.80	0.62	0.54	0.59	0.19	0.60	0.21	0.37	4.24
Mean	13.68	0.37	0.92	0.67	0.68	0.65	0.21	0.68	0.26	0.40	4.84
Oats											
Larain	15.06	0.43	0.98	0.63	0.47	0.77	0.14	0.87	0.22	0.45	4.96
Brighton	13.44	0.59	0.88	0.64	0.62	0.79	0.18	0.84	0.22	0.41	5.17
Legacy	12.88	0.36	0.78	0.56	0.34	0.63	0.12	0.74	0.18	0.39	4.10
Ajax	12.50	0.47	0.78	0.60	0.56	0.72	0.14	0.70	0.16	0.33	4.46
Exeter	12.44	0.36	0.74	0.54	0.37	0.59	0.13	0.72	0.17	0.35	3.97
Eagle	10.50	0.47	0.65	0.51	0.45	0.62	0.13	0.62	0.15	0.29	3.89
Mean	12.81	0.45	0.80	0.58	0.47	0.69	0.14	0.75	0.18	0.37	4.43
Barley											
Newal	12.75	0.36	0.73	0.54	0.53	0.58	0.17	0.52	0.18	0.36	3.97
Sanalta	12.19	0.34	0.69	0.52	0.49	0.55	0.17	0.51	0.17	0.32	3.76
Titan	12.12	0.37	0.79	0.47	0.56	0.65	0.22	0.60	0.24	0.45	4.35
Olli	11.62	0.36	0.67	0.48	0.44	0.52	0.16	0.51	0.17	0.31	3.62
O.A.C. 21	11.25	0.37	0.70	0.43	0.49	0.60	0.19	0.57	0.19	0.38	3.92
Montcalm	10.56	0.37	0.67	0.42	0.49	0.54	0.19	0.56	0.19	0.37	3.80
Mean	11.75	0.36	0.71	0.48	0.50	0.57	0.18	0.54	0.19	0.37	3.90

Referring to the examples mentioned above, it may be noted from the data in Table XVIII that although the proteins of the malting varieties Olli, O.A.C. 21 and Montcalm were found to contain a higher proportion of lysine than the proteins of the three feed varieties the amount of lysine in the whole grain was, due to the fact that the feed varieties contain a higher percentage of protein, essentially the same in all six varieties. Similarly, in the samples assayed, the total percentage of nine amino acids in whole grain is shown to be higher in the wheat samples than in the barley or oat samples, despite the fact that the wheat proteins contained a smaller proportion of these amino acids than did the barley or oat proteins. The final evaluation of the nutritional significance of data such as these, derived by the microbiological amino acid method, must be based on carefully planned and executed animal feeding trials.

The results of the present study and data previously reported (22) from this laboratory indicate that, in general, as the protein content of cereal grains increases the proportion of lysine in the protein tends to decrease. Thus a cereal grain breeding program, designed to attempt to combine in one variety genetic factors which contribute to high protein with factors which are associated with high proportions of essential amino acids, might be fraught with difficulty. Nonetheless, in view of the extreme importance in human and livestock rations of protein rich in essential amino acids, it would seem that possibilities of this nature may be worthy of thorough investigation.

SUMMARY

1. The results of microbiological assays for nine essential amino acids in six varieties of wheat, oats and barley have been reported.

2. The variability in per cent of total nitrogen in all three grains was found to be highly significant for both varieties and stations.

3. In each species the fraction of the total nitrogen contributed by the nitrogen of lysine was found to decrease with increasing protein.

4. The fraction of the total nitrogen contributed by the nitrogen of nine essential amino acids was found to be approximately 34.4% in oats, 31.7% in barley and 30.8% in wheat.

5. Differences attributable to both variety and station have been found to be statistically significant for the proportions of lysine, valine and arginine in cereal grain proteins. The specific varietal and station comparisons made have been tabulated in each instance.

6. Differences, attributable to variety, in the essential amino acid content of grain proteins were not so marked in wheat as in oats or barley.

7. As measured by the proportion of the single amino acid lysine or by the proportion of the nine essential amino acids

studied, the oat varieties Ajax, Eagle and Brighton (hulless) appeared to contain proteins of somewhat better quality than those of the Larain, Legacy or Exeter varieties.

8. Similarly in barley, the varieties Titan, O.A.C. 21 and Montcalm may be characterized by proteins of better quality than those of Newal, Sanalta or Olli varieties. The variety Titan appears not only to contain protein of somewhat superior quality but has the additional advantage of a comparatively high protein content.

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APPENDIX

APPENDIX

ASSAY TECHNIQUE

Preparation of Samples

Composite samples obtained from each station, for each variety listed in Table I, were taken from the 1946 crop of wheat, oats and barley. Samples were prepared for assay by grinding through the 1 mm. screen of a Wiley mill. Ground samples were stored in glass bottles at room temperature.

Hydrolysis

In all assays conducted, one gram of the finely ground sample was used. The weighed samples were transferred to Erlenmeyer flasks and 25 ml. of 2 N hydrochloric acid were added. The Erlenmeyer flasks, covered with 50 ml. beakers, were placed in the autoclave and subjected to live steam for 10 hours at 15 pounds pressure.

Neutralization and Dilution

Since nine amino acids were assayed concurrently it was found that an assay set-up using nine unknowns in each assay was of suitable size.

The hydrolyzed samples were transferred from the Erlenmeyer flasks and diluted to 100 ml. in graduated cylinders. Filtration through coarse filter paper (No. 1) removed the coarse particles of humin formed and facilitated subsequent pipetting.

Twenty-five ml. were removed from each hydrolysate and brought to pH 6.8 with concentrated solutions of sodium hydroxide and acetic acid. A Beckmann potentiometer equipped with external electrodes and a stirrer was used for neutralization. The neutralized solution was made up to a final volume of 50 milliliters. This dilution of 1 g. in 200 ml. was used for the assay of lysine.

A 20 ml. aliquot of the 1 g. in 200 ml. dilution was diluted with 30 ml. of distilled water. This dilution of 1 g. in 500 ml. was used for assaying leucine, isoleucine, valine, methionine, arginine, histidine and threonine.

A 10 ml. aliquot of the 1 g. in 500 ml. dilution was diluted with 10 ml. of distilled water to make a dilution of 1 g. in 1000 ml. for assaying phenylalanine.

All dilutions were made up in pairs and assays were run at three different levels of unknown. Unknowns were pipetted into 20 x 150 mm. Pyrex test tubes. The levels of the unknown samples added to each tube were 0.4, 0.6 and 0.8 milliliters. Distilled water was added previously to the tubes at corresponding levels of 0.6, 0.4 and 0.2 ml. to make a volume of 1 ml. in each tube. Unknowns were added with a 1 ml. Mohr pipette whereas distilled water was pipetted into tubes with an automatic dispenser designed by Cannon (15).

Standard Solutions

Stock solutions of each amino acid assayed were prepared at an exact concentration of 0.5 mg. per milliliter. The amounts of each amino acid used in the stock solution, together with the

dilution made and the range covered are shown in Table 1.

Table 1
Standard Solutions

Amino Acid	Mg. for 200 ml. stock solution	Dilution of stock solution	Range covered
1 lysine.HCl.H_2O	138	20 ml. to 250 ml.	0-40 microgram
1(-) leucine	100	10 " " " "	0-20 "
dl isoleucine	200	10 " " " "	0-20 "
dl phenylalanine	200	5 " " " "	0-10 "
dl valine	200	10 " " " "	0-20 "
dl methionine	200	5 " " 500 "	0- 5 "
1(+) arginine.HCl.	121	10 " " 250 "	0-20 "
1(+) histidine.HCl.H_2O	135	15 " " 500 "	0-15 "
dl threonine	200	5 " " 250 "	0-10 "

The standard solutions, made up for each assay, were adjusted to pH 6.8 before dilution to the required concentration as specified in Table 1. The standard solutions were included in each assay at the following levels: 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml. Distilled water was added to each tube at corresponding levels of 1.0, 0.8, 0.6, 0.4, 0.2 and 0.0 ml. to make a volume of 1 ml. in each tube. Each level of the standard solution used was run in triplicate so that an assay for one amino acid employed 72 tubes, 18 for the standards and 54 for the unknowns. Metal test tube racks, designed to hold ten rows of six tubes each and provided with metal covers, were used to handle the tubes.

Basal Media

Basal media for each amino acid assay were made up fresh from the stock solutions listed in Table 2. The basal media used in all assays were similar to those used by Riesen, Clandinin,

Elvehjem and Cravens (28). As in their method:

1. The amino acids were included at the maximum levels in order to standardize the media for the three organisms employed.
2. Pyridoxamine was used exclusively in place of pyridoxine. It has been reported by Snell and Rannefeld (38) that it is doubtful if lactic acid bacteria can utilize unchanged pyridoxine.
3. Xanthine was included in all media.
4. Choline and inositol were omitted.

The basal media differed from those used by Riesen _et_ _al_. (28) in that proline was included in the media for the other organisms as well as for _Leuconostoc_ _mesenteroides_ P-60. In addition methionine assays were conducted with a synthetic medium in which pure amino acids were used to replace the acid hydrolysate of oxidized casein employed by the Wisconsin workers. The amounts of amino acids used in the methionine basal medium employed in this study were standardized to the same level as in media for each of the other eight amino acids.

Stock solutions, used to make up the basal media, were stored in the refrigerator at a temperature of approximately 4°C. The solutions, together with the method of preparation, are listed in Table 2. Anhydrous C.P. glucose, sodium acetate and sodium citrate were weighed out as required.

Table 2
Composition of Stock Solutions for Basal Media

I Amino Acid Solution - 120 milliliters

l leucine	0.5 grams
dl isoleucine	1.0 "
dl valine	1.0 "
l(-) cystine	1.0 "
dl methionine	1.0 "
dl tryptophane	0.5 "
l(-) tyrosine	0.5 "
dl phenylalanine	0.5 "
l(+) glutamic acid	2.0 "
dl threonine	1.0 "
dl alanine	1.0 "
dl asparagine	2.0 "
dl lysine.HCl.H_2O	2.0 "
l(+) arginine.HCl	0.5 "
l(+) histidine.HCl.H_2O	0.5 "
dl serine	1.0 "
l(-) proline	0.25 "
glycine	0.5 "

Nine separate solutions were made up. The amino acid under assay in each solution was omitted.

Dissolve amino acids in 10-12 ml. concentrated HCl and a small quantity of water by heating.

Each solution was made up to a total volume of 120 milliliters.

II Salts A - 100 milliliters

KH_2PO_4	10 grams
K_2HPO_4	10 "

Dissolve by warming in 100 ml. distilled water.

III Salts B - 100 milliliters

$MgSO_4.7H_2O$	4.0 grams
$FeSO_4.7H_2O$	0.25 "
$MnSO_4.4H_2O$	0.25 "
NaCl	0.25 "

Dissolve by warming in 100 ml. distilled water.

IV Purines and Pyrimidines - 100 milliliters

Adenine sulfate.$2H_2O$	0.1 grams
Guanine.HCl.$2H_2O$	0.1 "
Uracil	0.1 "

Dissolve in a few drops of HCl by heating. Dilute with 100 ml. distilled water.

V Xanthine - 100 milliliters

Xanthine	100 milligrams

Dissolve in small quantity of concentrated NH_4OH and make up to 100 ml. with distilled water.

Table 2 (continued)

VI Vitamin Solution - 250 milliliters

Thiamine hydrochloride	12.5	mg.
Pyridoxamine dihydrochloride	5.0	"
dl-calcium pantothenate	12.5	"
Riboflavin	12.5	"
Niacin	25.0	"
Para-aminobenzoic acid	2.5	"
Biotin	25	γ
Folic acid	250	γ

Dissolve by heating in 250 ml. distilled water. Use NH_4OH to dissolve folic acid and $HC_2H_3O_2$ to dissolve riboflavin.

Each solution was stored in the refrigerator at a temperature of approximately 4°C.

The amounts of each of the constituents to make up sufficient basal media for 100 assay tubes is shown in Table 3.

Table 3
Composition of Basal Media

Ingredient	Amount Used for 100 Tubes
Glucose	4.0 grams
Sodium acetate(1)	4.0 "
Sodium citrate	5.0 "
Salts A	1.0 ml.
Salts B	1.0 "
Purines and Pyrimidine	2.0 "
Xanthine	2.0 "
Amino Acid Solution	5.0 "
Vitamin Solution	2.5 "

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(1) Sodium acetate was used as a buffer in the assay of lysine, leucine, isoleucine, phenylalanine and valine, whereas sodium citrate was used in the assay of methionine, arginine, histidine and threonine.

The glucose and buffer salts were added to approximately 50 ml. of distilled water and brought into solution by heating. The appropriate amounts of the stock solutions as listed in Table 3 were added and the resulting mixture was adjusted to pH 6.8 with brom thymol blue indicator. The final volume was made to 100 ml. with distilled water.

One ml. of the appropriate basal medium was pipetted into each of the assay tubes by means of a Cannon automatic dispenser to give a final volume of 2 milliliters.

Stock Cultures

Three organisms were used to assay nine amino acids in the

unknown samples. The organisms were the same as those employed by Riesen et al. (28) and were obtained from the American Type Culture Collection, Georgetown University, Washington, D.C. Stock cultures of the organisms were carried on a medium of the composition listed in Table 4.

Table 4
Composition of Stock Culture Medium

Ingredient	Quantity for 100 ml.
Salts B solution (Table 3)	0.5 ml.
K_2HPO_4 solution (.1 g. per ml.)	0.5 "
Vitamin solution (Table 3)	2.0 "
Glucose	2.0 g.
Tryptone	1.0 "
Yeast extract	0.5 "
Sodium acetate	1.0 "
Agar	1.5 "

The tryptone, yeast extract and agar were obtained from Difco Laboratories, Detroit, Michigan.

The cultures were preserved in a refrigerator at a temperature of approximately 4°C. and were transferred at intervals of two weeks.

Inoculation, Incubation and Titration

To grow the inoculum for the assay tubes a transfer was made from each stab culture into a tube containing 5 ml. of medium of the same composition as that listed in Table 4 excepting agar was omitted. This liquid culture was incubated for 24 hours at 37°C. and the cell suspension so obtained was packed by centrifugation. The supernatant was decanted and the bacteria resuspended

in 30 ml. of 0.9 per cent sterile saline. This suspension constituted the inoculum.

The tubes, containing a volume of 2 ml., were autoclaved at 15 pounds pressure for 12 minutes, cooled to room temperature and inoculated with one drop of inoculum. After 72 hours incubation in a forced air incubator at 37°C. the contents of each tube were titrated to pH 6.8 with 0.1 N sodium hydroxide solution using an automatic recording titrator (15). The early assays conducted in this study were titrated with a burette equipped with a titrating device designed by Lunardelli (20). The standard curve was plotted for each assay from the titration values. The final amino acid content of each sample was based on the average values for the three levels of assay.

Calculations

The standard curve drawn from the results for the tubes containing fractions of the standard solutions covered the ranges denoted in Table 1. In the case of the lysine assay the standard curve covers a range of 0 - 40 micrograms. Thus 1 ml. of the standard solution exhibited the lysine activity of 40 micrograms l(-) lysine. If 1 ml. of a 1 g. in 200 ml. dilution of the unknown hydrolysate was found to promote the same growth as 0.5 ml. of lysine standard it was assumed to contain the equivalent of 20 micrograms l(-) lysine. Thus 1 gram of the original sample would contain 4 mg. l(-) lysine.

The assay values reported as per cent amino acid nitrogen of total nitrogen were calculated from these figures.

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